# THE DYNAMICS OF CELLULASE SYNTHESIS IN FUSARIUM CULTURES: INFLUENCE OF CELLULOSE CONCENTRATION IN THE CULTURE MEDIA.

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*Summary*

Cellulase synthesis is not only controlled by the structure of cellulose (Targoński, Szajer, 1979), but also by its concentration in the culture medium. The activity of endo- glucanase and aryl-β-glucosidase increases with increasing concentration of cellulose, whereas the activity of cellobiose dehydrogenase may also depend on other factors. The latter enzyme may facilitate the formation of lactones in culture media, which are known to act as inhibitors of cellulases.

## *Introduction*

The dominant goal of all biotechnological processes is to increase the yield of the synthesized end-products, i.e. biomass, enzymes, amino acids etc. One of the important factors which influence those processes is the concentration of substrates in the culture media. In the particular case of cellulase synthesis it is known that exceeding the concentration of 0.75 - 1.25% of cellulose in culture media usually caused a decrease in cellulolytic activity, irrespective of the type of cellulose used as substrate for the culture.

The present investigation aims at specifying some aspects of this problem, especially the relation between cellulose concentration in the culture media and cellulase biosynthesis, by a Fusarium isolate.

*Materials and Methods*

1. Cellulose substrates: cotton, filter paper, cotton pretreated with 85% $H_3PO_4$ (cotton "H").

2. The microorganism and culture media were the same as described in our previous paper (1979) with the exception that the concentration of cellulose was varied between 0.5% and 6.0%, and the concentrations of mineral salts in the media were increased in the same proportion. The cultures were maintained on a rotary shaker at 28°C, usually until the highest endo-glucanase activity was attained. At thatstage the culture media were centrifuged (20 min at 1,500 x g). The supernatant was used for enzyme assay and the residue, several times washed with water, was analysed for dry matter and protein content (Hofsten, Ryden, 1975). The amounts of protein in culture media were estimated by the Lowry method.

3. Enzyme assays: the activity of endo-glucanase and the ability of culture filtrates to hydrolyse native cotton were measured as described in the previous paper (1977). The activity of cellobiose dehydrogenase was estimated following the Westermark-Eriksson method (1974), using 2,6-dichlorophenolindophenol as substrate.

*Results*

The experiments in which the effect of cellulose concentration in culture media on the activity of cellulolytic enzymes was studied, were carried out using the three cellulose substrates listed above. It can be seen from the curves in figures 1, 2 and 3, that increasing the cellulose concentration in the culture media causes an increase in endo-glucanase activity. The highest activity (and the longest time to maximum activity) were obtained when native cotton was used as the carbon source (fig. 2.). Considerably lower activities of endo-glucanase were found in culture media after growing the Fusarium isolate in media enriched with filter paper (fig. 1). The lowest activity was found after growing the fungus in media containing cotton "H", and in this case the maximum activity was attained in the shortest time (see fig. 3).

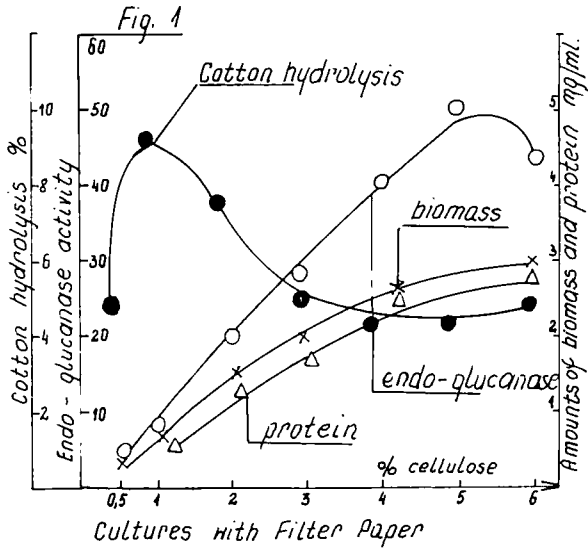
However, the ability of culture filtrates to hydrolyse native cotton was also dependent on the amount of cellulose used in the culture media, but in a different manner. When cultures contained over 1% of cellulose (filter paper or native cotton) or over 2% (when cotton "H" was used), the hydrolytic activity of culture filtrates tested decreased significantly (see figs. 1, 2 and 3).

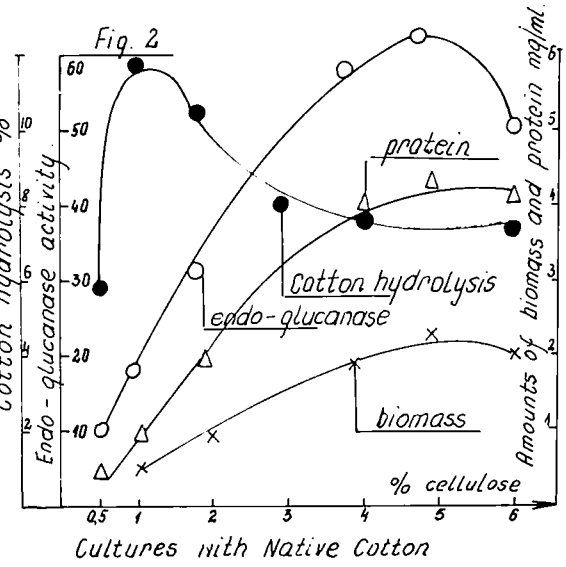
The activity of cellobiose dehydrogenase in the enzyme complex synthesized by the Fusarium isolate was also estimated. This activity was also found to increase with increasing amounts of cellulose in the culture media. The highest activities of cellobiose dehydrogenase were found in culture filtrates after growth with filter paper as the only carbon source (fig. 4). The culture filtrates obtained after the growth of the Fusarium on cotton "H" and on native cotton showed significant differences in their endo-glucanase activities but similar levels of cellobiose dehydrogenase activity. It would be interesting to find out what kind of mechanisms control the biosynthesis of the cellobiose dehydrogenase enzymes.

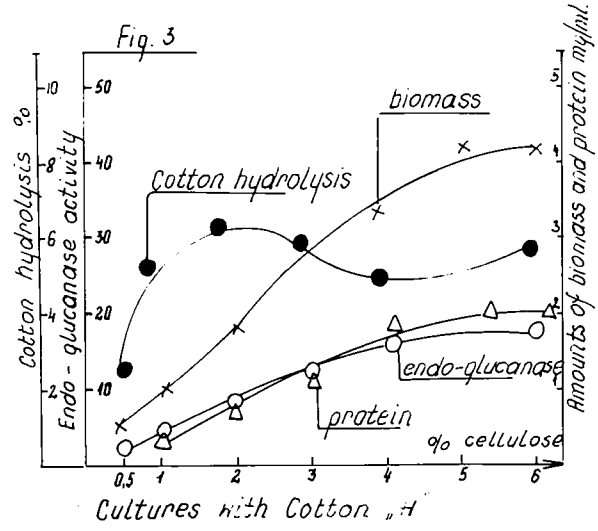
The amount of biomass as well as the level of the protein in culture filtrates were measured in addition to the estimates of enzyme activity. Increased amounts of both protein and biomass were found in the cultures grown on higher cellulose concentrations. The highest amounts of protein were found in culture media from the growth of Fusarium on native cotton, although the amount of biomass showed its lowest value (fig. 2.) Opposite results (highest biomass and lowest protein) were obtained after the growth of Fusarium on cotton "H" (fig.3.)

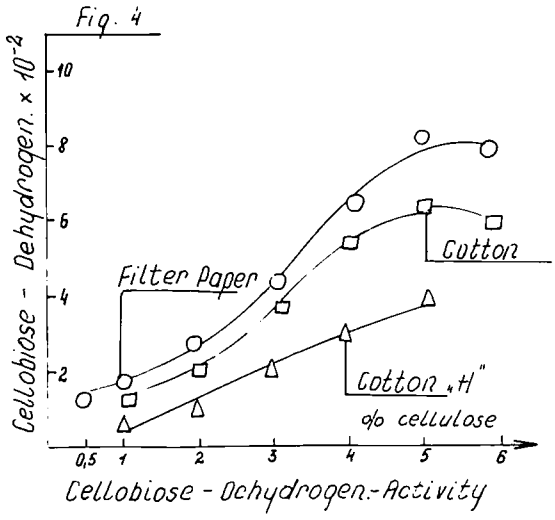
When the cellulose content in the media exceeded 1 or 2%, the culture filtrates showed diminished hydrolytic activity against cotton. Changes in hydrolytic activities following simple dilution of culture filtrates were investigated after culturing the fungus with 2 and 3% additions of cellulose. Table 1 shows that dilution of the culture filtrates obtained after the growth of Fusarium isolate on 2 and 3% cellulose media causes a 10 - 70% increase in activity. This fact has an

Influence of cellulose concentration in culture media on the activity of cellulases and cellobiose-dehydrogenase




| The hydrolytic acitivity of culture filtrates against cotton after the growth of Fusarium sp. in media with 2 and 3% of cellulose. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Type of cellulose used as "C" | Hydrolytic activity as % of hydrolyzed cotton | | | | | | | |
| | cultures with 2% cellulose | | | | with 3% of cellulose | | | |
| | not diluted | | 2xdiluted | | not diluted | | 3xdiluted | |
| Filter paper | 7.90 | 100% | 8.70 | 110% | 5.0 | 100% | 8.5 | 170% |
| Native cotton | 10.3 | 100% | 12.30. | 116% | 8.2 | 100% | 12.0 | 147% |

important bearing on any discussion of the possible reasons for the lowering of cellulose hydrolysis, when higher levels of cellulose are provided in the culture media and apparently higher levels of the relev ant enzymes are being formed.

*Discussion*

The generally low yield in cellulase biosynthesis is one of the important factors which limit the utilization of cellulases on a large scale. It is known that when the concentration of cellulose substrates in the growth media exceeds 0.7 to 1.3% a decrease in cellulolytic activity is regularly observed (Ghose, Pathak and Bisario, 1975; Mandels and Weber, 1969). In connection with the results obtained in the present series of experiments we can nevertheless conclude that the activity of endo-glucanase and the activity of cellubiose dehydrogenase, as well as the amounts of soluble protein and the production of biomass, were not adversely influenced by increasing the amounts of cellulose in the media (figs 1, 2 and 3). We would therefore suggest that the observed decrease of the cotton hydrolysis activity at higher levels of cellulose in the growth media is perhaps explained by the increased activity of cellobiose dehydrogenase (fig.4). High activity of this enzyme is very likely to cause a corresponding accumulation of lactones in the reaction mixtures, and such lactones are known to inhibit the cellulases (Westermark and Eriksson, 1974). It should also be remembered that the hydrolysis of cotton was measured by incubations carried out at 50°C. during 24 hours, whereas the endo-glucanase activity was measured at 30°C. during 20 min. Hence there exists the possibility that the concentration of the inhibitors was too low to react actively during the shorter time, but it was high enough to inhibit the hydrolysis of cotton over 24 hours. This explanation certainly seems to be confirmed by the results obtained when diluted or undiluted culture filtrates were compared for their activity in the hydrolysis of native cotton (table 1).

*References*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